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Filed on 18 June 2002 (18.06.2002)

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(74) Agent: **HAILE, Lisa, A.**; Gray Cary Ware & Freidenrich LLP, 4365 Executive Drive, Suite 1100, San Diego, CA 92121-2133 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF SCREENING FOR AGENTS THAT MODULATE IMMUNOPHILIN/PEPTIDYLPROLINE CIS-TRANS ISOMERASE (PPIASE)-HOMER INTERACTION

(57) Abstract: The invention features a method of identifying, evaluating and screening for compounds or agents for the treatment of disorders involving the Homer signaling pathway in the modulation of immunosupression and neuroprotection. The method inlcudes evaluating the ability of agents to modulate Homer protein activity, Homer protein/immunophilin-peptidylproline cis-trans isomerase interaction, and/or Homer protein/proline-type Homer ligand consensus sequence interaction to identify agents for such treatment. The invention also discloses treatment modalities involving agents identified by such methods.

WO 2005/007844 A1

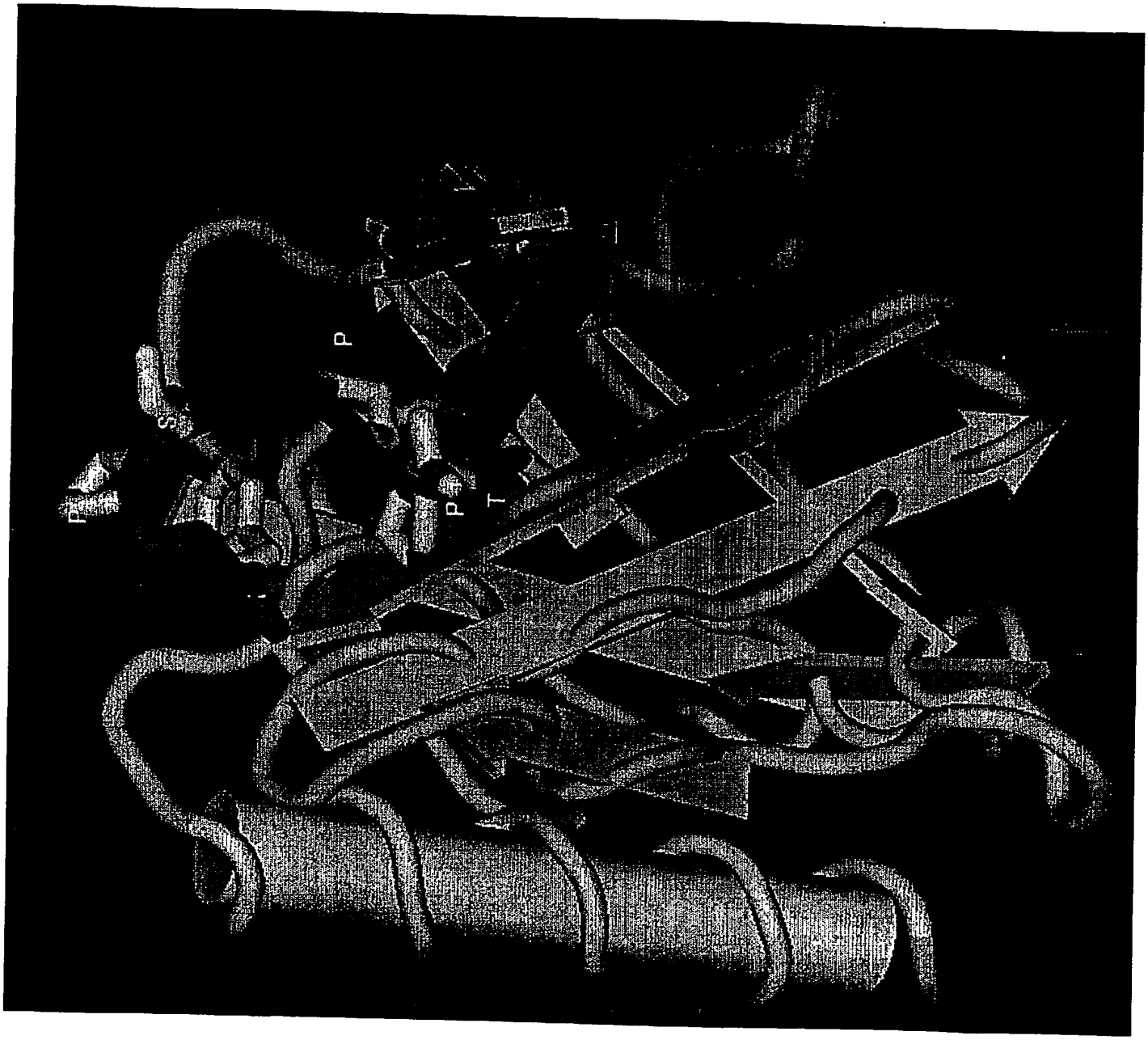


FIGURE 1

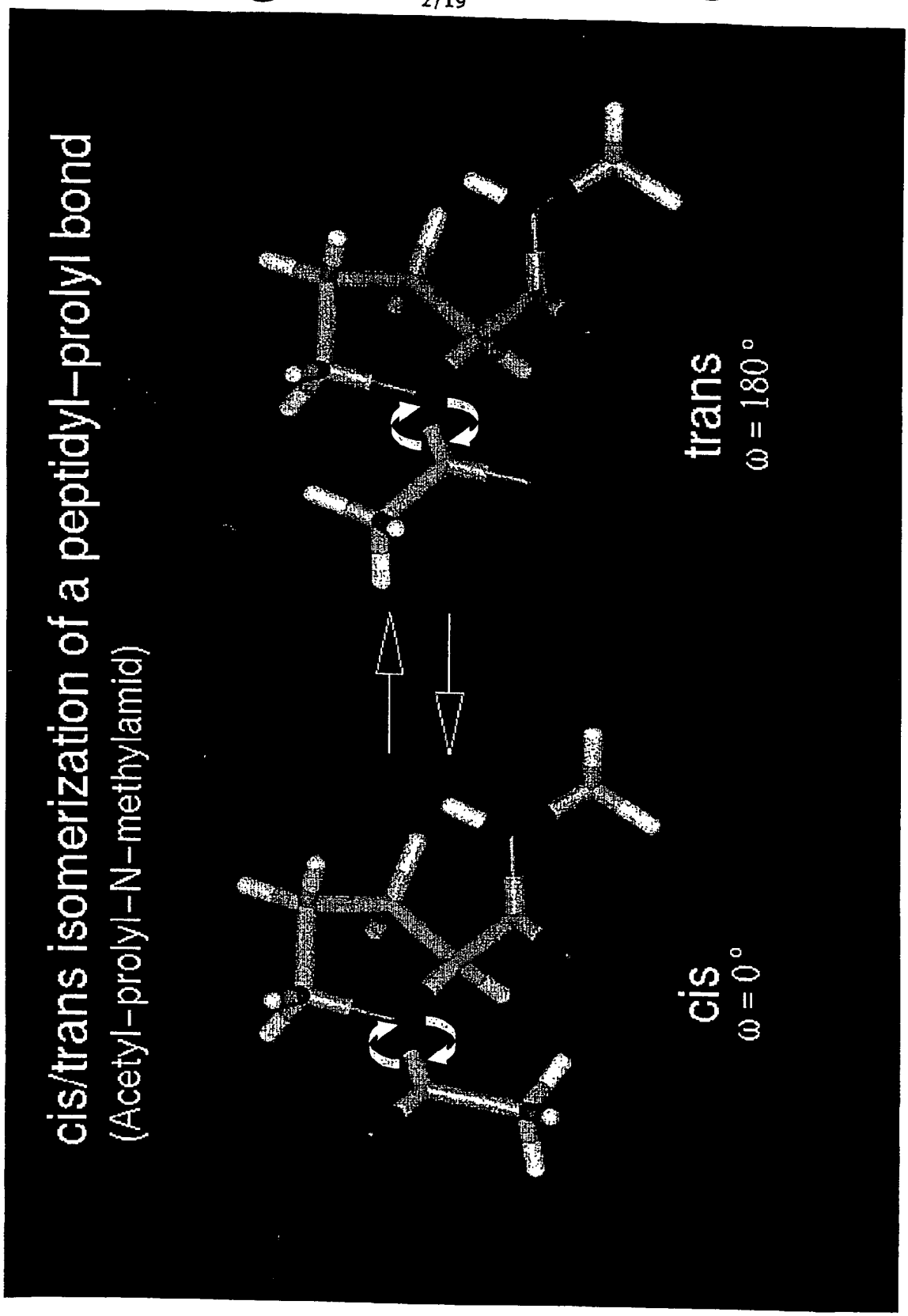
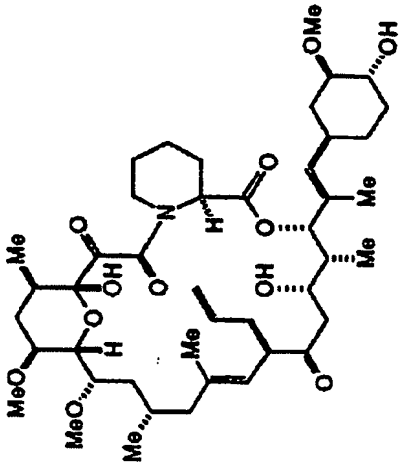
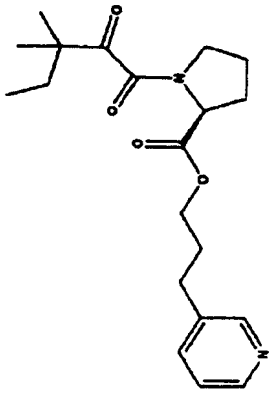


FIGURE 2

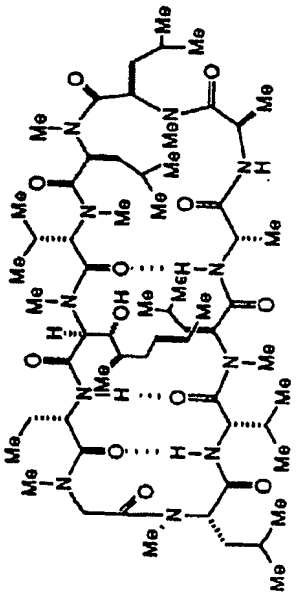
Structure of Immunophilin Ligands



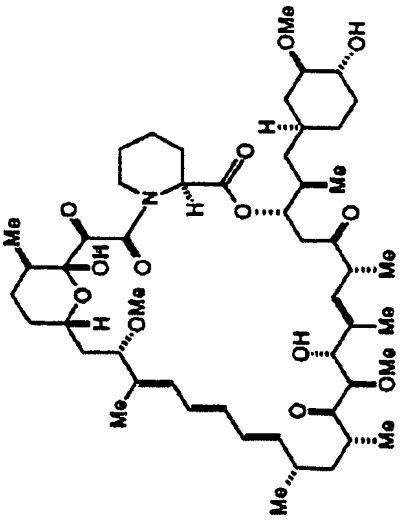
FK506



GPI-1046



Cyclosporin A

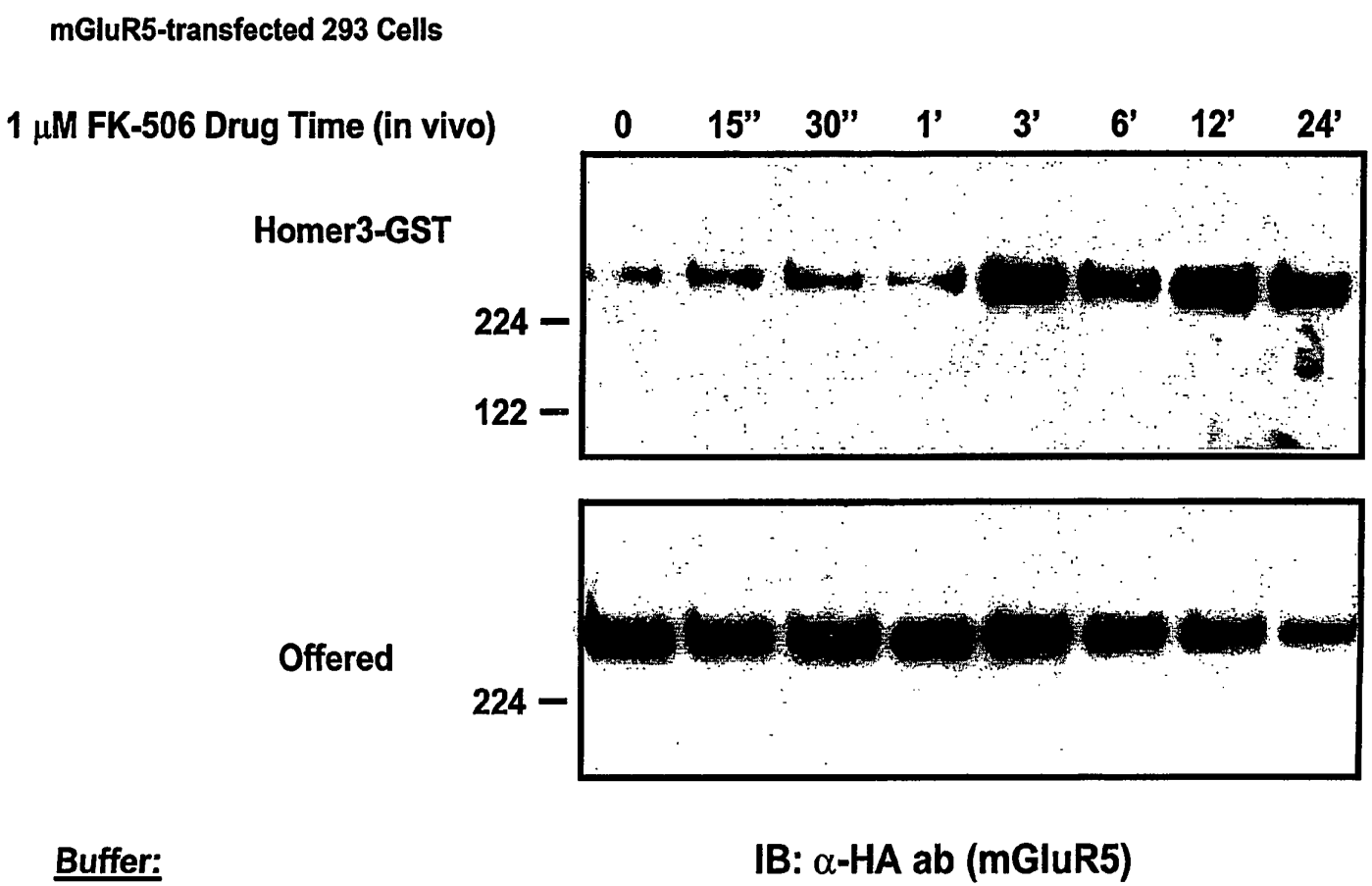


Rapamycin

FIGURE 3

Effect of FK-506 on mGluR5 Binding to Homer

Time Course

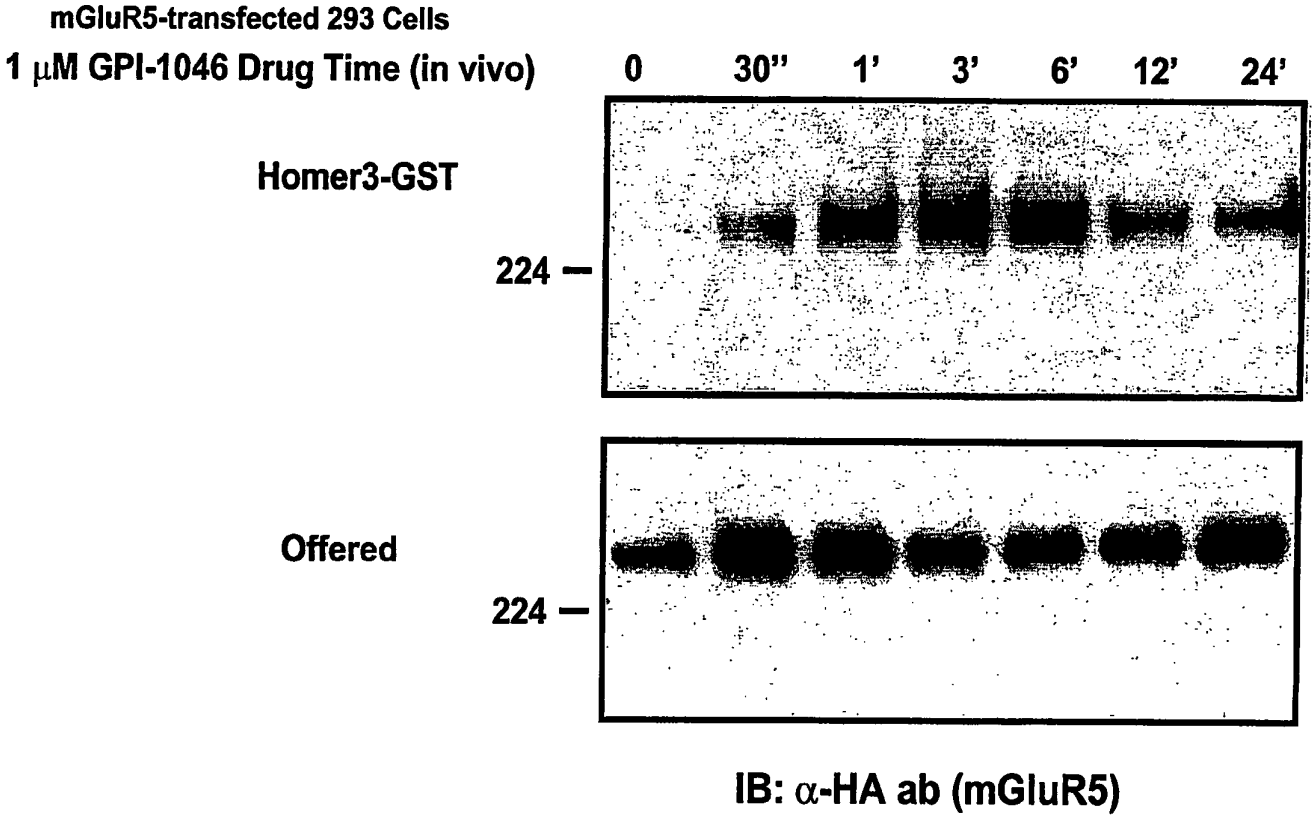


Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

FIGURE 4

Effect of GPI-1046 on mGluR5 Binding to Homer

Time Course



Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

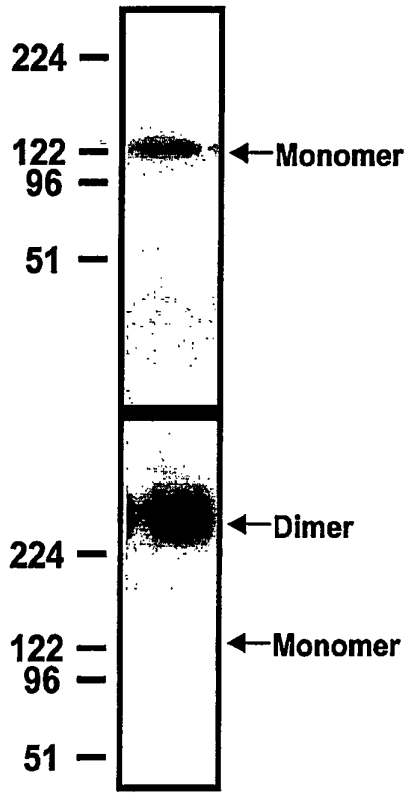
FIGURE 5

FKBP52-GST Pulls Down mGluR5

mGluR5-transfected 293 Cells

Homer 3-GST

Offered



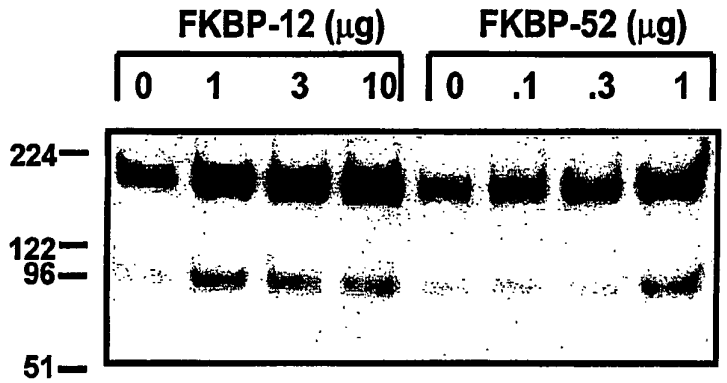
Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

IB: α-TRPC1 ab

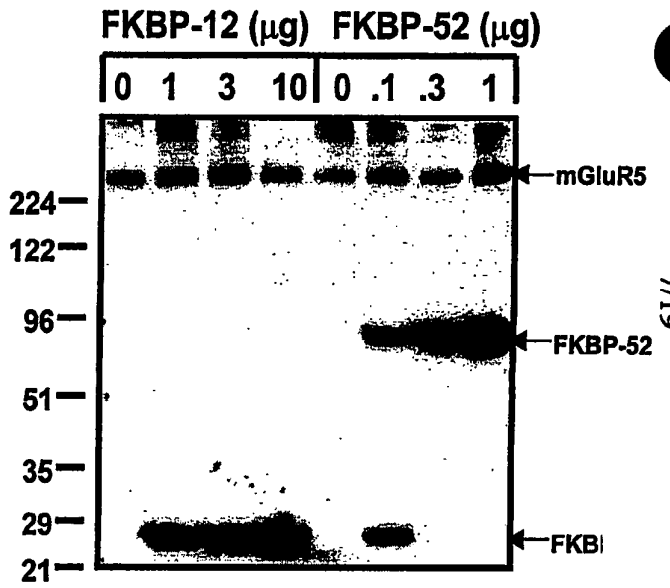
FIGURE 6

Increasing FKBP-12/-52 *Increases* Homer Binding to mGluR5

mGluR5-transfected 293 Cells +:



Homer3-GST



Offered

Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

IB: α-HA ab

FIGURE 7

Phylogenetic Relationship in the TRP Protein Family

Clapham et al., 2001

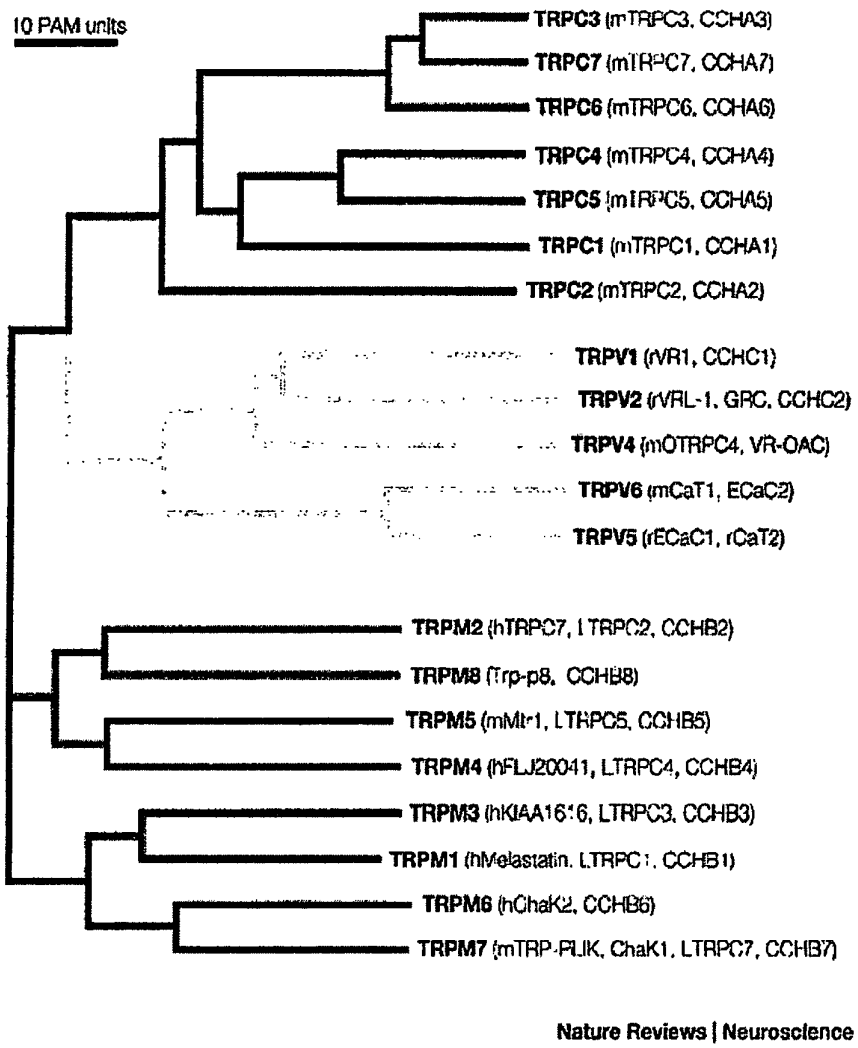


FIGURE 8

Amino Acid Sequence of TRPC1 and Alignment to Other TRPs

Wes et al., 1995

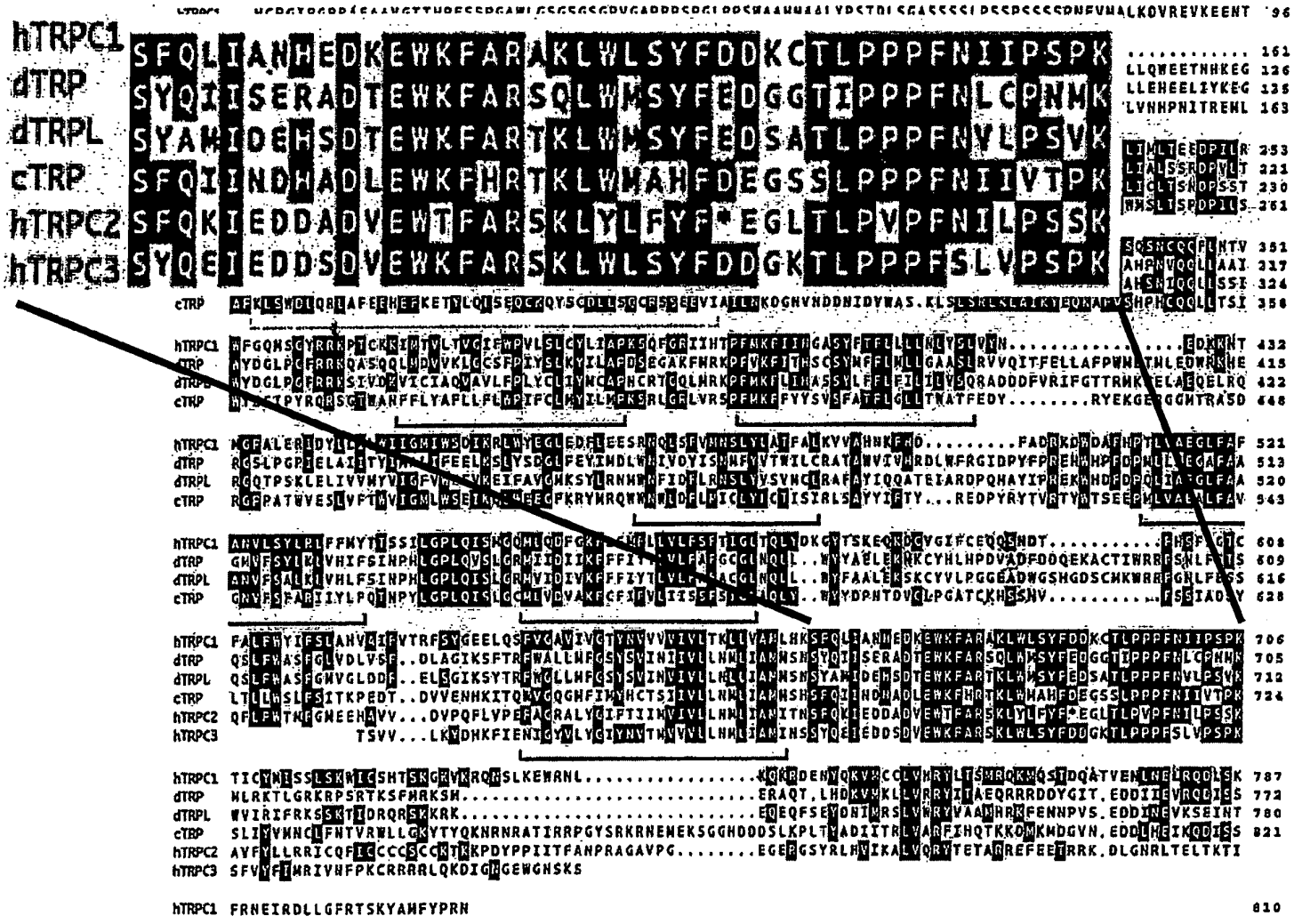


FIGURE 9

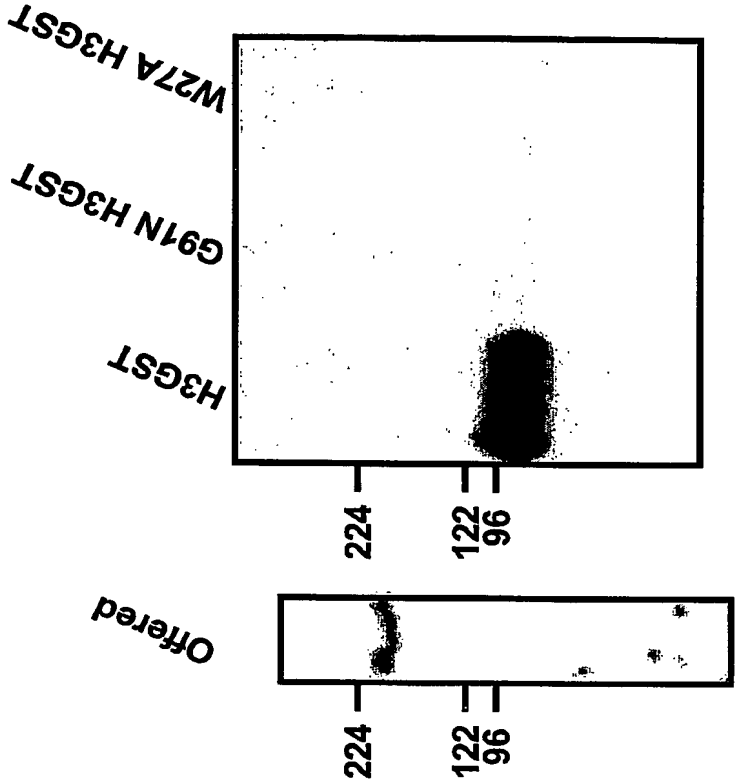
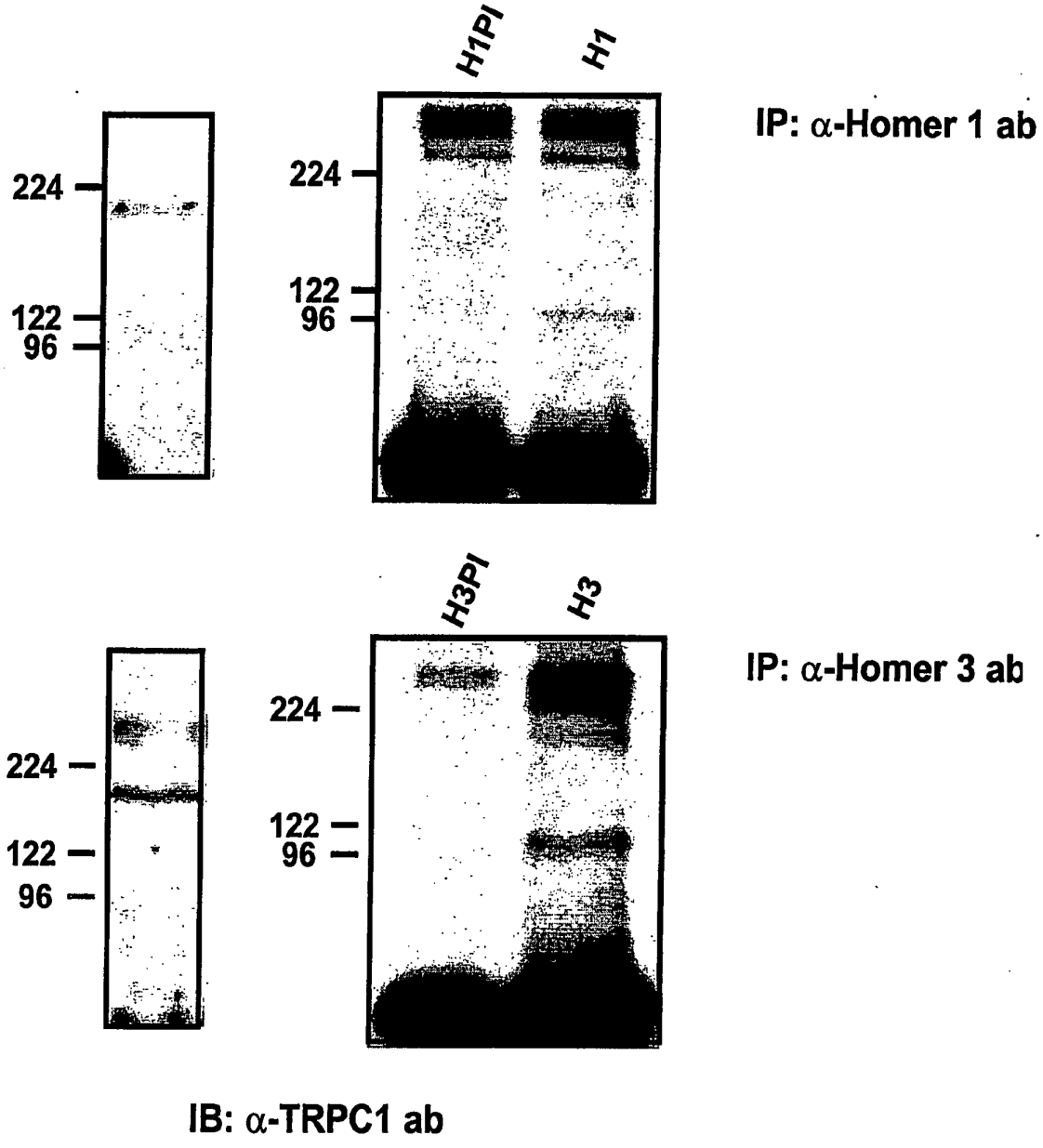

Homer 3-GST Pulls Down TRPC1 from Cerebellum
Solubilized in 1 % TX-100
37,000 x g Spin
Buffer:
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor
Offered
H3GST
G91N H3GST
W27A H3GST
224
122
96
224
122
96
IB: α-TRPC1 ab

FIGURE 10

Brain Co-IP of Homer and TRPC1



Hippocampus

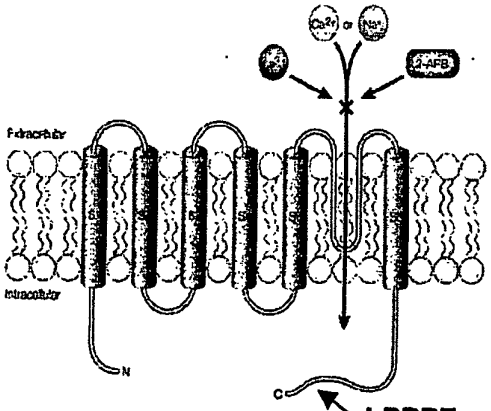
Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

Cerebellum

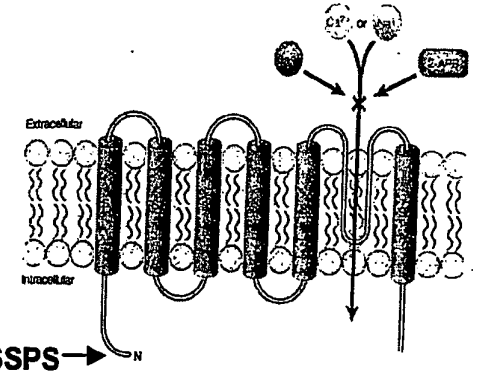
Buffer:
1 % CHAPS
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

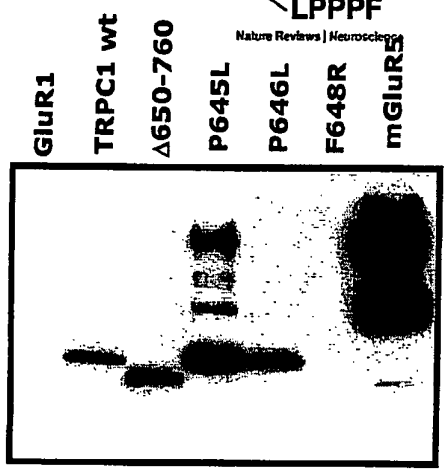
FIGURE 11

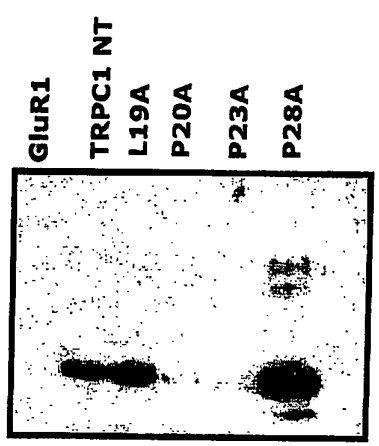
Deletion of TRPC1 C-Terminus Reveals A Novel Homer Binding Site at the N-Terminus

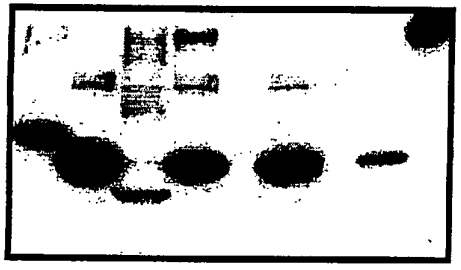


C-Terminal Deletion









IB: α-HA ab (TRPC1)

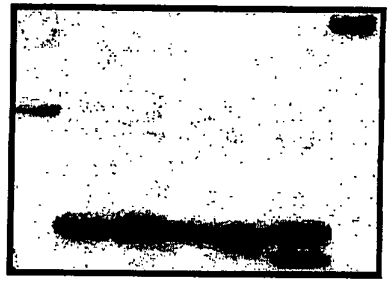


FIGURE 12

WT and G91N Homer-GST Binds To LPSSP Motif of TRPC1 N-Terminus

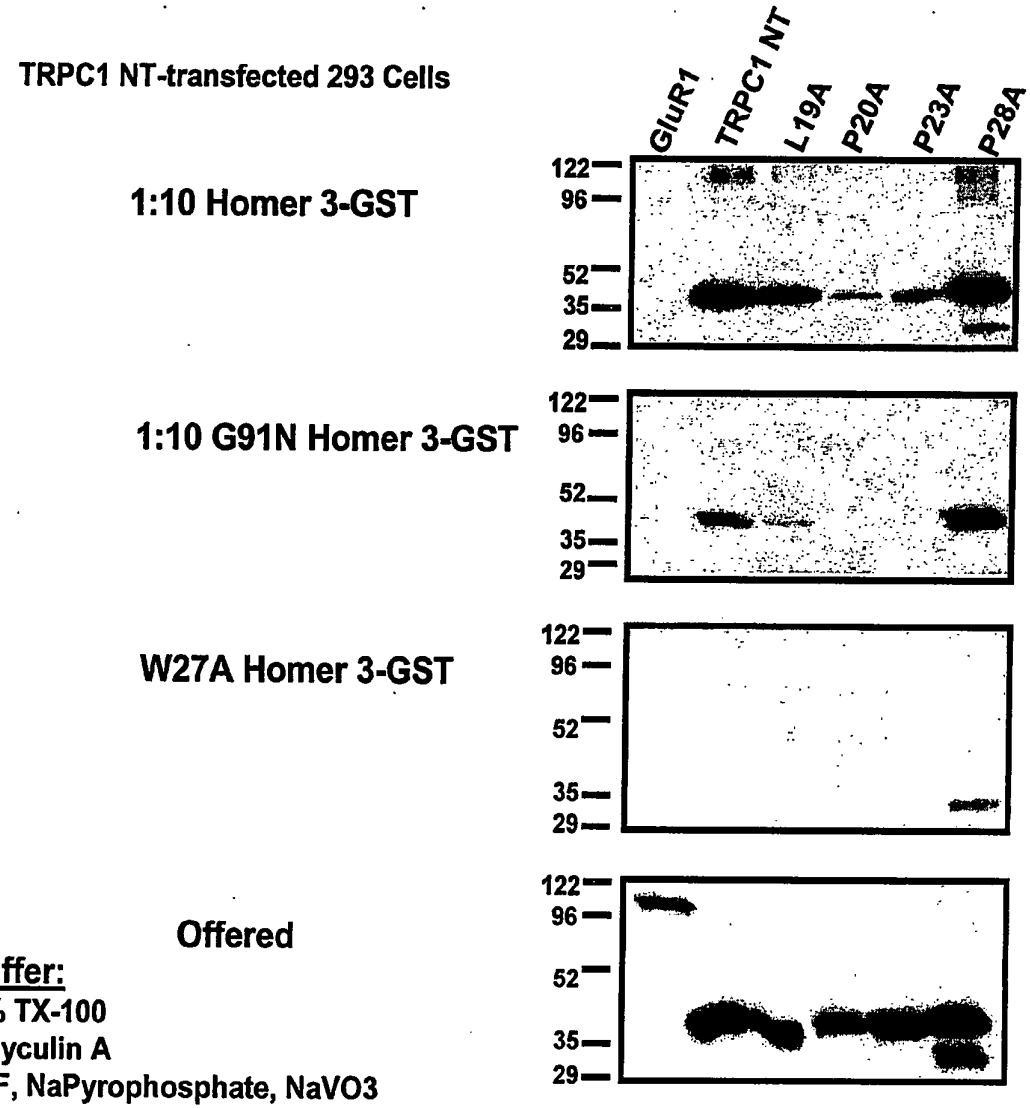
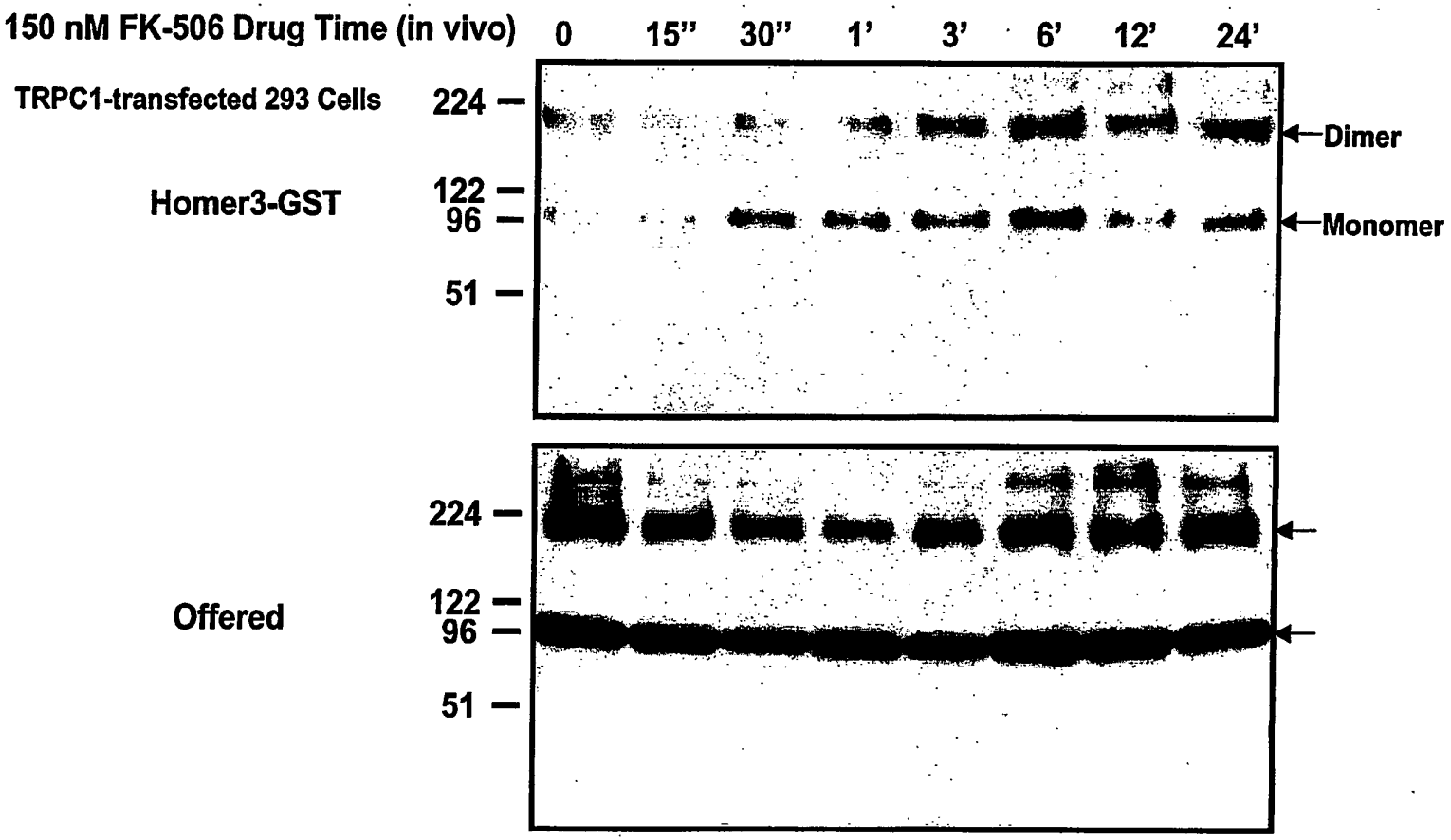


FIGURE 13

Effect of FK-506 on TRPC1 Binding to Homer

Time Course



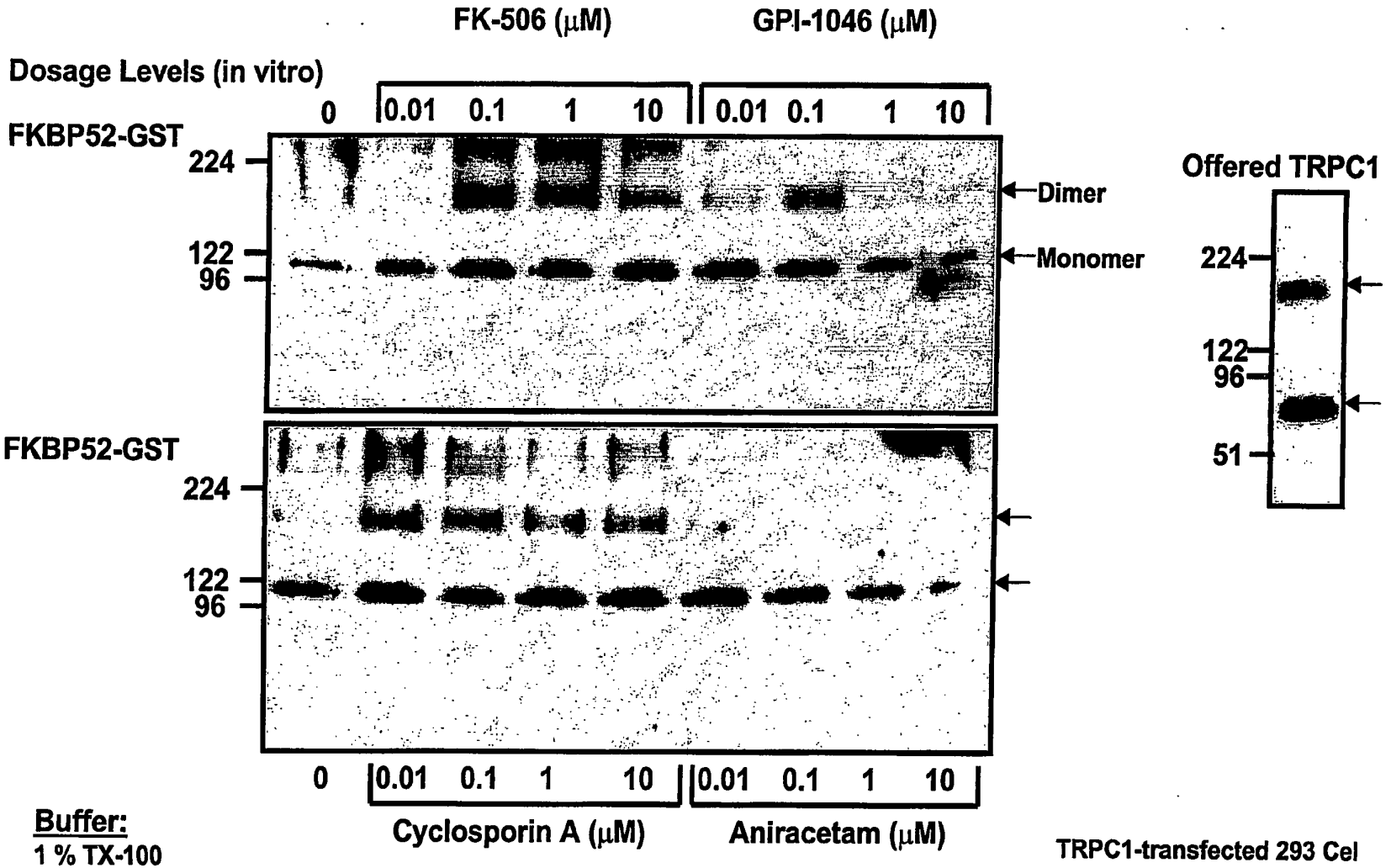
Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

IB: α-HA ab (TRPC1)

FIGURE 14

Effect of Various Drugs on TRPC1 Binding to FKBP52

Dosage Dependence



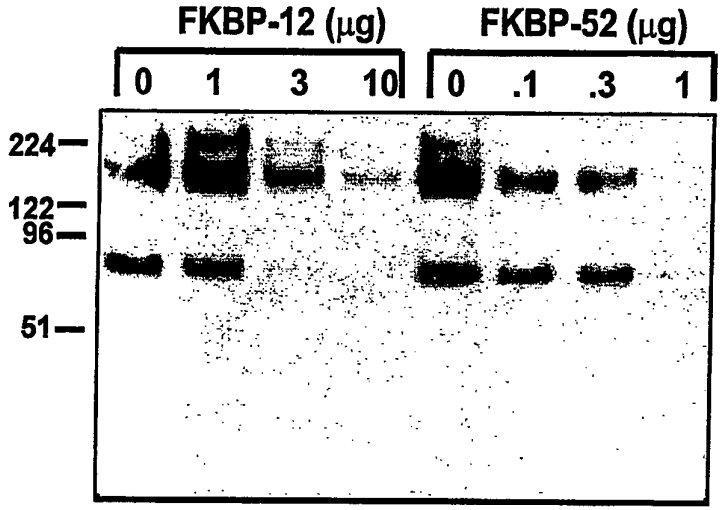
Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

IB: α-HA ab (TRPC1)

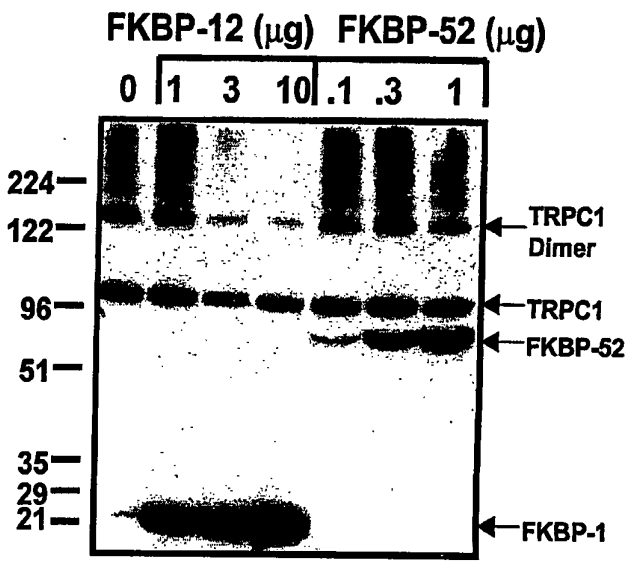
FIGURE 15

Effect of FKBP-12/FKBP-52 on TRPC1 Binding to Homer

TRPC1-transfected 293 Cells +:



Homer3-GST



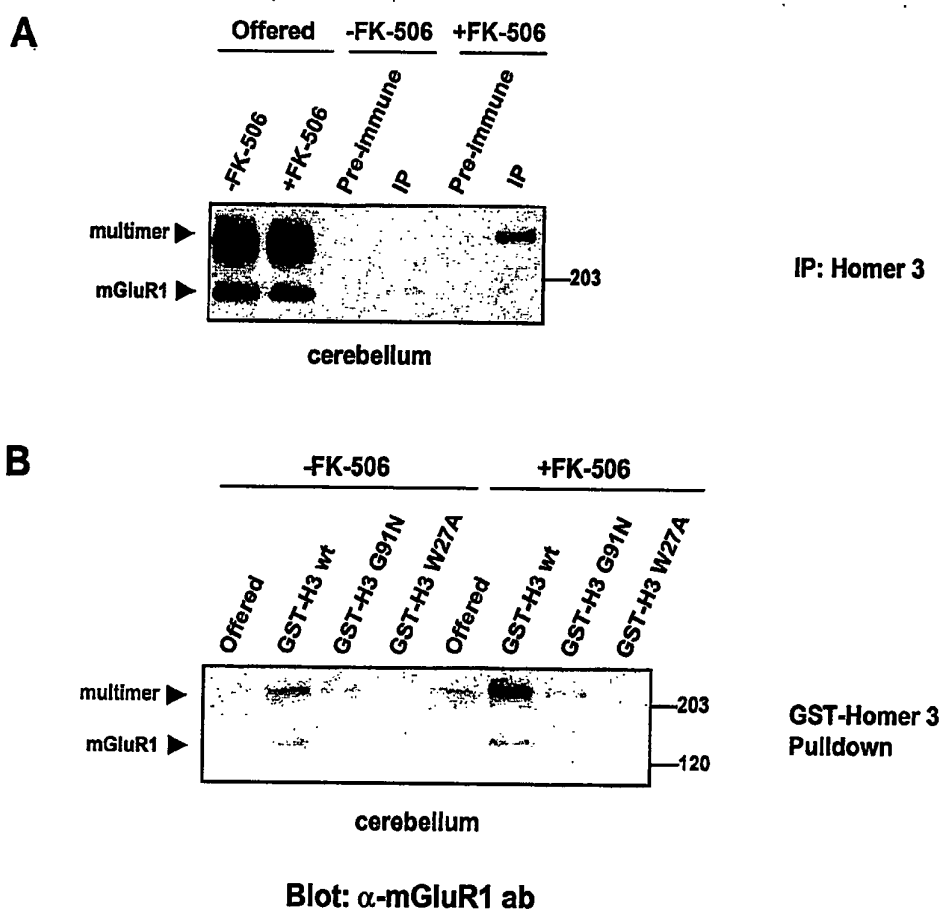
Offered

Buffer:
1 % TX-100
Calyculin A
NaF, NaPyrophosphate, NaVO3
Protease Inhibitor

IB: α-HA ab (TRPC1)

FIGURE 16

Effect of FK-506 on Homer-mGluR1 interaction *in vivo*



Adult rat was injected (i.p.) with 10 mg/kg FK-506 or vehicle only (DMSO). After 3 hours, the rats were sacrificed, and the cerebellum was collected. (A) Homer 3 co-immunoprecipitation of mGluR1 multimer increases with FK-506 treatment vs. control. Rats +/- FK-506 express equal offered amounts. (B) WT GST-Homer 3 pulldown of mGluR1 multimer increases with FK-506 treatment vs. control.

FIGURE 17

```
atg ggg gaa caa cct atc ttc agc act cga gct cat gtc ttc cag atc
gac cca aac aca aag aag aac tgg gta ccc acc agc aag cat gca gtt
act gtg tct tat ttc tat gac agc aca agg aat gtg tat agg ata atc
agt cta gac ggc tca aag gca ata ata aat agc acc atc act cca aac
atg aca ttt act aaa aca tct caa aag ttt ggc caa tgg gct gat agc
cgg gca aac act gtt tat gga ctg gga ttc tcc tct gag cat cat ctc
tca aaa ttt gca gaa aag ttt cag gaa ttt aaa gaa gct gct cgg ctg
gca aag gag aag tcg cag gag aag atg gaa ctg acc agt acc cct tca
cag gaa tca gca gga gga gat ctt cag tct cct tta aca cca gaa agt
atc aat ggg aca gat gat gag aga aca ccc gat gtg aca cag aac tca
gag cca agg gct gag cca gct cag aat gca ttg cca ttt tca cat agg
tac aca ttc aat tca gca atc atg att aaa
```

Figure 18

Met Gly Glu Gln Pro Ile Phe Ser Thr Arg Ala His Val Phe Gln Ile
Asp Pro Asn Thr Lys Lys Asn Trp Val Pro Thr Ser Lys His Ala Val
Thr Val Ser Tyr Phe Tyr Asp Ser Thr Arg Asn Val Tyr Arg Ile Ile
Ser Leu Asp Gly Ser Lys Ala Ile Ile Asn Ser Thr Ile Thr Pro Asn
Met Thr Phe Thr Lys Thr Ser Gln Lys Phe Gly Gln Trp Ala Asp Ser
Arg Ala Asn Thr Val Tyr Gly Leu Gly Phe Ser Ser Glu His His Leu
Ser Lys Phe Ala Glu Lys Phe Gln Glu Phe Lys Glu Ala Ala Arg Leu
Ala Lys Glu Lys Ser Gln Glu Lys Met Glu Leu Thr Ser Thr Pro Ser
Gln Glu Ser Ala Gly Gly Asp Leu Gln Ser Pro Leu Thr Pro Glu Ser
Ile Asn Gly Thr Asp Asp Glu Arg Thr Pro Asp Val Thr Gln Asn Ser
Glu Pro Arg Ala Glu Pro Ala Gln Asn Ala Leu Pro Phe Ser His Arg
Tyr Thr Phe Asn Ser Ala Ile Met Ile Lys

Figure 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/19499

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/90;C12P 21/04;C07K 7/00, 17/00; A61K 38/00, 45/00, 39/00
US CL : 435/233, 70.1; 530/300, 350;514/2,12; 424/85.1, 198.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/233, 70.1; 530/300, 350;514/2,12; 424/85.1, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
caplus, biosis, issued patents, NPL

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed,USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,E	US 6,720175(WORLEY et al.) 13 April 2004 (04.13.2004), example 12 (column 46, line 49) and example13 (column 47, line15).	1-15, 18
A	BRECHT etal. Changes in Peptidlyl-prolyl cis/trans Isomerase Activity And FK506 Binding Protein Expression Following Neuroprotection By FK506 In The Ischemic Rat Brain. Neuroscience. 2003, Vol. 120, pages 1037-1048.	1-15, 18, 22-24, 27, 29, 31-34
A	BARTOLOMEIS et al.Acute Admistration of Antipsychotics Modulates Homer Striatal Gene Expression Differentially. Mol. Brain Res. 2002, Vol 98, pages 124-129, especially page 128.	31-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
16 November 2004 (16.11.2004)

Date of mailing of the international search report
13 DEC 2004

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Commissioner for Patents
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Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Gyan Chandra

Telephone No. (571)272-1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/19499

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 16,17,19-21,28 and 30
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
No paper sequence listing or computer readable form have been submitted.

3. ☒ Claim Nos.: 25 and 26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-15,18,22-24,27,29 and 31-34

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-24 and 27-34, drawn to a method of screening for modulating agents of a Homer signaling pathway.

Group II. Claims 35 and 38-40, drawn to a method of preserving nerve bundles after surgery by administering to a subject in need thereof a therapeutic amount of a pharmaceutical composition comprising an agent identified by the method of Group I.

Group III. Claims 36 and 37, drawn to a method of modulating sensory perception by administering to a subject in need thereof a therapeutic amount of a pharmaceutical composition comprising an agent identified by the method of Group I.

Group IV. Claims 41-45, drawn to a method of treating a neurological disorder by administering to a subject in need thereof a therapeutic amount of a pharmaceutical composition comprising an agent identified by the method of Group I.

Group V. Claims 46 and 47, drawn to a method of inducing immunosuppression or treating inflammation by administering to a subject in need thereof a therapeutic amount of a pharmaceutical composition comprising an agent identified by the method of Group I.

Group VI. Claims 48 and 49, drawn to a method of treating hematological disorders by administering to a subject in need thereof a therapeutic amount of a pharmaceutical composition comprising an agent identified by the method of Group I.

Group VII. Claims 50-65, drawn to a method of diagnosing a homer signaling disorder.

Group VIII. Claims 66-71, drawn to a method of determining the efficacy of a PPIase inhibitor.
13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I-VIII are drawn to completely different methods each having completely different method steps, using different compositions, and having completely different outcomes. These methods are not interchangeable and which require non-cohesive searches and considerations.

The special technical feature of Group I is considered to be a method of screening for modulating agents of a Homer signaling pathway.

The special technical feature of Group II is considered to be a method of preserving nerve bundles after surgery by administering to a subject in need thereof an agent that modulates a homer signaling pathway.

The special technical feature of Group III is considered to be a method of modulating sensory perception by administering to a subject in need thereof an agent that modulates a homer signaling pathway.

The special technical feature of Group IV is considered to be a method of treating a neurological disorder by administering to a subject in need thereof an agent that modulates a homer signaling pathway.

The special technical feature of Group V is considered to be a method of inducing immunosuppression or treating inflammation by administering to a subject in need thereof an agent that modulates a homer signaling pathway.

The special technical feature of Group VI is considered to be a method of treating hematological disorders by administering to a subject in need thereof an agent that modulates a homer signaling pathway.

The special technical feature of Group VII is considered to be a method of diagnosing a homer signaling disorder.

The special technical feature of Group VIII is considered to be a method of determining the efficacy of a PPpase inhibitor.

Accordingly, Groups I-VIII are not so linked by the same or a corresponding special technical feature as to form a single general concept.

INTERNATIONAL SEARCH REPORT

PCT/US03/194[redacted]

In the absence of any response from the applicant, this Authority will establish the International Search Report based on the main invention. The claims drawn to the main invention are as follows:

Claims 1-24 and 27-34, drawn to a method of screening for modulating agents of a Homer signaling pathway.